MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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I. Introduction

During this quarter we continued our examination of multielectrode activation of hindlimb extensors and flexors. By using two or more (up to four) stimulating electrodes, positioned in or near the motoneuron pool of the hindlimb extensors, a variety of complex interactions occurred. These interactions include additive, synergistic, and inhibitory responses as well as changes in response fatigue. These interactions were highly dependent on electrode separation and stimulus parameters. Some of these interactions have been reported in previous progress reports. Some new findings during this quarter include: (1) the use of different frequencies of stimulation applied to each of a pair of electrodes can reduce the amount of responses of fatigue; (2) interleaving of stimuli is an effective means of enhancing a response when electrodes are separated by at least 1.5 to 2 mm and continues to be effective at a separation of 3 mm (largest separation tested); (3) very small changes in frequency (1 or 2 Hz) of one of a pair of electrodes can dramatically change the response fatigue.

Also during the quarter we began some new studies to determine the anatomical location and distribution of neurons and interneurons in the spinal cord which modulate colon activity. These studies utilized pseudorabies virus as a tracer and indicate that colon neurons are located in the lumbosacral spinal cord medial and dorsal to the bladder preganglionic neurons. The details of these studies are reported below.

II. Hindlimb Flexion and Extension Elicited by Multielectrode Activation

The purpose of these studies was to examine the effects of multielectrode activation of motoneuron pools on the extension torque produced by the hindlimb about the knee joint. The idea being that the use of more than one stimulating electrode could produce extension torque at reduced current density and possibly with a decrease in the amount of response fatigue. Furthermore that electrode separation and specific stimulus parameters could also contribute to

enhanced torque responses.

The techniques used have been described in detail in previous progress reports. Extension torque is record from the cat hindlimb to microstimulation of the L6 spinal cord. A fixed array of four electrodes are used for microstimulation. The electrodes are oriented rostrocaudally, parallel to the motoneuron pools. Figures 1, 2, and 3 show the responses along three tracts (1, 2, and 3) of four electrodes (A, B, C, and D). Tract 1 is the most lateral and is located just at the dorsal root entry zone of the L6 segment. Tract 2 is at the same level but 0.3 mm medial while tract 3 is 0.3 mm medial to tract 2. The four stimulating electrodes are each separated by 1 mm from the adjacent electrodes. Electrode A is the most rostral and D the most caudal. Figures 1, 2, and 3 show the individual responses at different depths (from surface to 4.2 mm) for each electrode along three different mediolateral tracts. Notice that flexion torque is produced at or a few mm from the surface of the spinal cord, while extension torque is produce deep within the ventral horn. Since the electrode array is fixed and moves as a unit along the vertical axis the response cannot be maximized along each electrode tract. This can be seen in Figures 1, 2, and 3 where the response is not always maximized at a given depth. In tract 1 (Figure 1) for example, the extension torque is small on two (C and D) of the four electrodes and only maximal for electrode B.

Once the individual responses from each electrode have been established the electrodes are activated in various combinations. An observation reported in a previous progress report and now confirmed in three additional animals is the enhanced response seen with interleaved stimulation when the distance separating each electrodes is increased above approximately 1.5 mm. Figure 4 shows the effects of interleaving with increasing distance of separation. The top two panels (A and B) in Figure 4 show examples of interleaving on two adjacent electrodes with 1 mm separation. Simultaneous stimulation (0.0 interleave) produces the largest torque response, while even small amounts of interleave only reduced the response. As the distance between electrodes

is increased (C and D in Figure 4) the interleaving produces a greater response torque than with simultaneous stimulation. The separation of electrodes A and C is 2 mm and of A and D 3 mm. The maximum separation we have examined in these experiments is 3 mm. These studies indicate that interleaving can improve the torque response when stimuli are present to two electrodes separated by at least 1.5 mm but simultaneous simulation is the preferred mode with electrode separation of 1 mm or less.

Frequency of simulation also seems to play an important role in multielectrode stimulation. In one series of experiments the stimulus frequency was kept constant on one electrode while varying the frequency of stimulation on the other electrode. Both extension torque and fatigue were recorded to a 30 second stimulus. Figure 5 shows this data for extension torque (top row Figure 5, A to D) and fatigue torque (bottom row Figure 5, E to G), for each of four frequencies (40, 30, 20, and 10 Hz) in all combinations. The highest torque is produced with both electrodes stimulated at 40 Hz (Figure 5 A). However the greatest fatigue (reduction in extension torque at the end of a 30 second stimulation) is also seen with both electrodes stimulated at 40 Hz (Figure 5 E). The best combination would be two frequencies which produce the largest extension torque (Figure 5 top row) and the smallest fatigue torque (Figure 5 bottom row). This occurs with electrode B at 10 Hz and A at 30 Hz (Figure 5 D and H) with both electrodes at 20 Hz (Figure 5 C and G) and also with A at 40 Hz and B at 10 Hz (Figure 5 A and E). These studies would suggest that certain combinations of frequencies can produce large extension torque with minimal or no fatigue.

Figure 6 also shows that even small differences of 2 or 3 Hz can greatly change the amount of fatigue generated by activation of two electrodes. The top panel in Figure 6 shows extension torque generated by two adjacent electrodes stimulated and the same (40 Hz) or slightly different (40 Hz ± 9 Hz) frequencies. The extension torque does not change dramatically but fatigue torque decreases dramatically when the stimuli are applied at some small frequency less

that synchronized. If one examines the type of stimulation being presented to the motoneuron pool the combination is quite complex. The stimuli are presented in a varying combination of small amounts of interleaving with simultaneous stimulation. These types of studies will continue into the next quarter, using combinations of three and four electrodes.

III. The Location and Distribution of Neurons and Interneurons Which Innervate the Colon

These studies were undertaken to determine the location in the lumbosacral spinal cord of neurons and interneurons which modulate the colon activity. The transynaptic tracer, pseudorabies virus (PRV) was used in these studies to determine the first order neurons as well as the interneurons in the multi synaptic pathway from spinal cord to colon. The anatomical location of these neurons will provide targets for future microstimulation experiments.

The general methods used have been reported in previous progress reports for other pelvic organs. A brief description of the methods unique to the colon injection are presented below.

Three immunologically naive male cats were anesthetized with halothane and the colon exposed using aseptic surgical procedures. Multiple (30 - 50) small volume (5 - 10 uL) injections of PRV (Becker strain) was injected into the colon wall. The injections were distributed from mid colon to within 6 cm of the anal opening. The distal end of the colon was avoided to prevent injections into the internal and external anal sphincters (IAS and EAS). The EAS and IAS will be studied in a separate group of experiments.

The virus was allowed to transport 80 - 96 hours and the animals perfused with Krebs solution followed by fixative. The spinal cord was removed, sectioned on a cryostat and processed with antibody to the PRV using standard techniques. The location of the PRV was visualized using fluorescent microscopy.

Since the colon, like other pelvic organs, receives innervation from both the

parasympathetic and sympathetic divisions of the autonomic nervous system, labeled neurons were observed in both the lumbar and sacral segments of the spinal cord (see Figure 7). Labeled preganglionic neurons were seen in the sacral parasympathetic nucleus (SPN) of the S2, S3, and caudal S1 spinal cord segments. Interneurons were seen lateral of the central canal and in the dorsal commisure of S1, S2, and S3, extending to rostral S1 (Figure 7, left). Labeling in the lumbar cord was seen in the intermediolateral cell column (IML). These neurons were sympathetic preganglionic neurons while neurons around the central canal and a few neurons in the dorsal commisure are likely interneurons.

Comparing these studies with our previous studies of the bladder, the colon preganglionic neurons are somewhat more medial and dorsal to those of the bladder. The colon interneurons seem somewhat less dense in the dorsal commisure than the bladder. The segmental distribution is however similar.

The penile preganglionic neurons, on the other hand, are slightly ventral to the colon preganglionic neurons and in general are densest in caudal S1 rather than S2 for the colon neurons. There seems to be some spacial segregation of excitatory neurons to the bladder, colon, and penis but some overlap probably exists. Microstimulation studies using these sites as targets will determine the amount of coactivation of these organs.

These types of studies will continue into the next quarter.

Figure 1. Line graphs showing the relationship between flexion or extension torque and depth in the L6 spinal cord for each of four electrodes (A, B, C, and D) of a fixed electrode array. Extension is shown as a torque in the negative direction. Stimulus parameters are 0.2msec pulse width, 40 Hz, 100uA, 10 seconds "on" 120 seconds "off". A is most rostral electrode; D is the most caudal in the spinal cord. The separation between electrodes is 1 mm. This first tract is at the dorsal root entry zone. Each additional tract is separated by 0.3mm, see Figures 2 and 3.

Figure 2. Same as Figure 1 except the electrode array has been moved 0.3 mm medial to tract #1 shown in Figure 1.

Figure 3. Same as Figures 1 and 2 except the electrode array has been moved 0.3 mm medial to tract # 2 shown in Figure 2.

Figure 4. Bar graphs showing the relationship between extension torque and the amount of stimulus interleave for each of 3 pairs of electrodes in two different tracts. Zero interleave is simultaneous stimulation. Adjacent electrodes are separated by 1 mm. Non-adjacent are separated by 1 mm increments: A and C = 2 mm separation; A and D = 3 mm separation. Stimulus parameters are: 0.2 msec pulse width, 40 Hz, uA as indicated on Figure, 10 seconds "on" 120 seconds "off". Notice that interleave produces enhanced torque when electrodes are separated by a greater distance, as shown in D.

Figure 5. Bar graphs showing the relationship between extension torque (top row A to D) or fatigue torque (bottom row E to H) and change in frequency to one electrode (A) of the pair of electrodes, while keeping the frequency constant to the other electrodes (B). All combinations are shown for both fatigue and extension torque. All stimulus parameters are the same except frequency. Stimulus parameters are: 100uA, 0.2 msec, 30 seconds "on" 120 second "off". Notice that with both electrodes delivering 40 Hz to the spinal cord site that the extension torque is maximal (A) but the fatigue torque is also maximal (E). However, if each site is stimulated at different frequency the extension torque may be somewhat reduced for example in (D) at 30 Hz but fatigue is nearly zero (H) at 30 Hz. Other frequency combinations also show reduced fatigue torque and good extension torque.

Figure 6. Bar graphs similar to Figure 5 showing the relationship between extension torque (top) or fatigue torque (bottom) and different frequencies delivered to one electrode while keeping the other electrode at a constant 40 Hz frequency. Notice the large reduction in fatigue torque with only a 3 Hz change in frequency to the other electrode.

Figure 7. Camera lucida drawing of section of the lumbar and sacral spinal cord showing the location and distribution of labeled preganglionic neurons and interneurons following injection of pseudorabies virus into the colon. S = sacral segments, L = lumbar segments, and r - rostral.

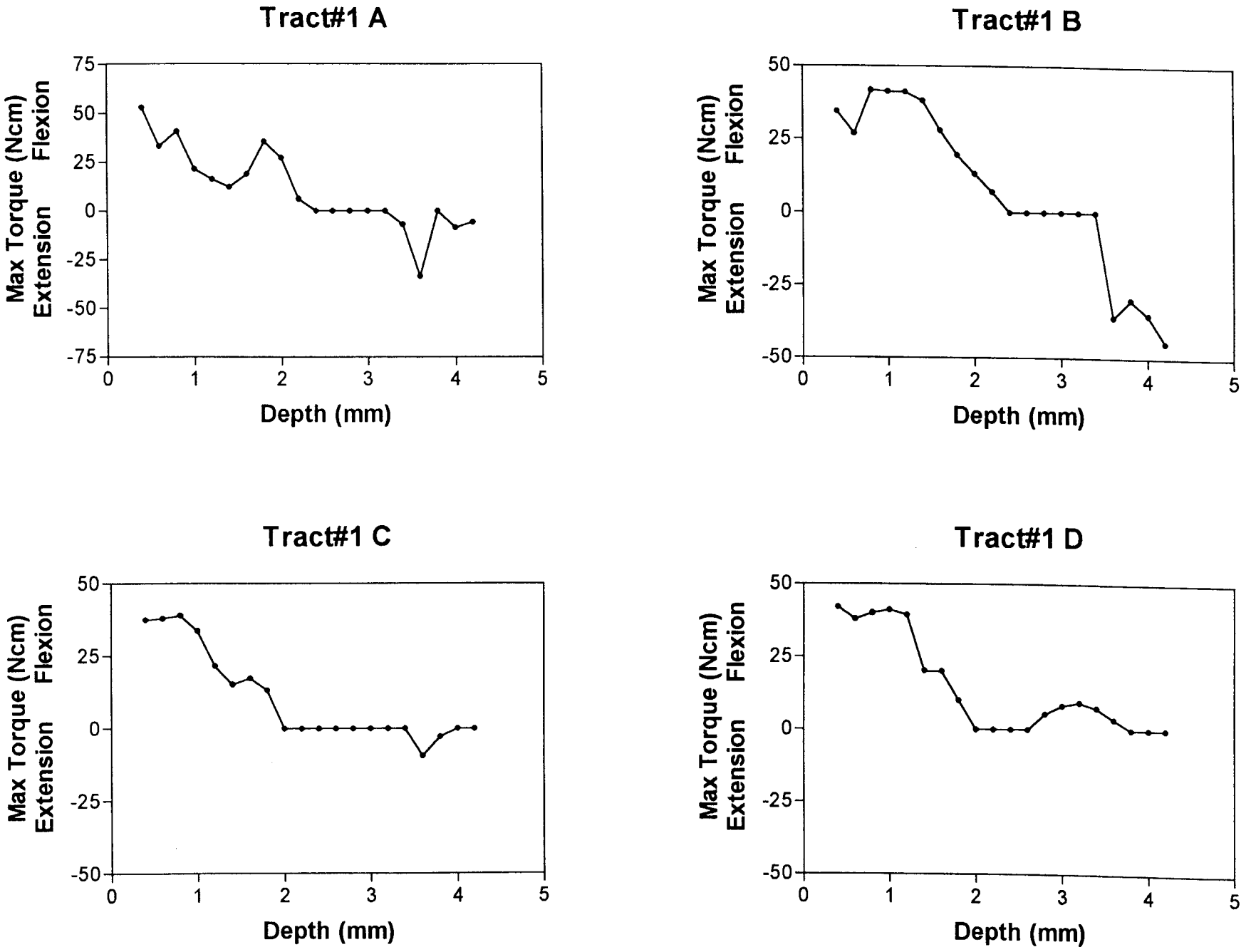
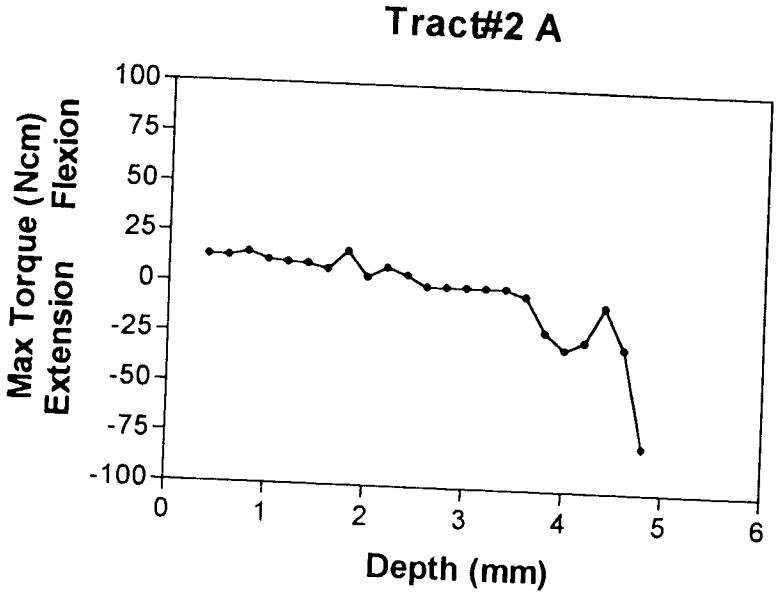
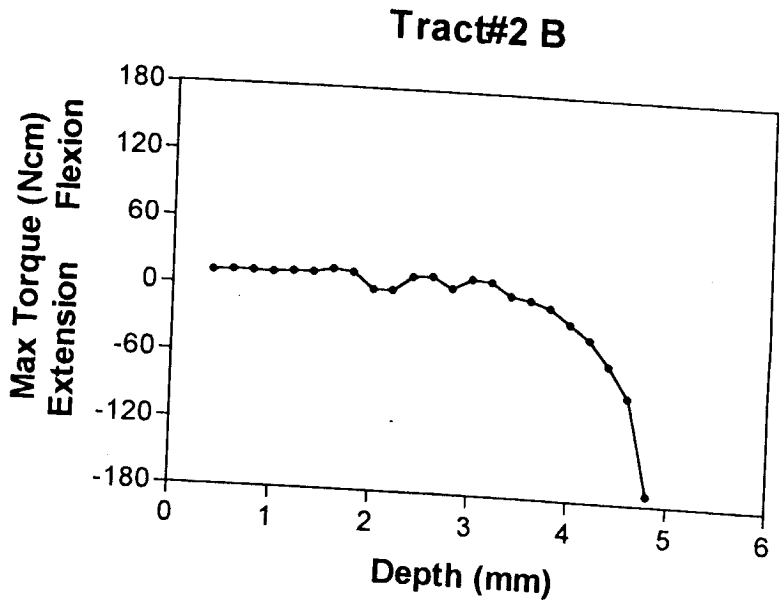
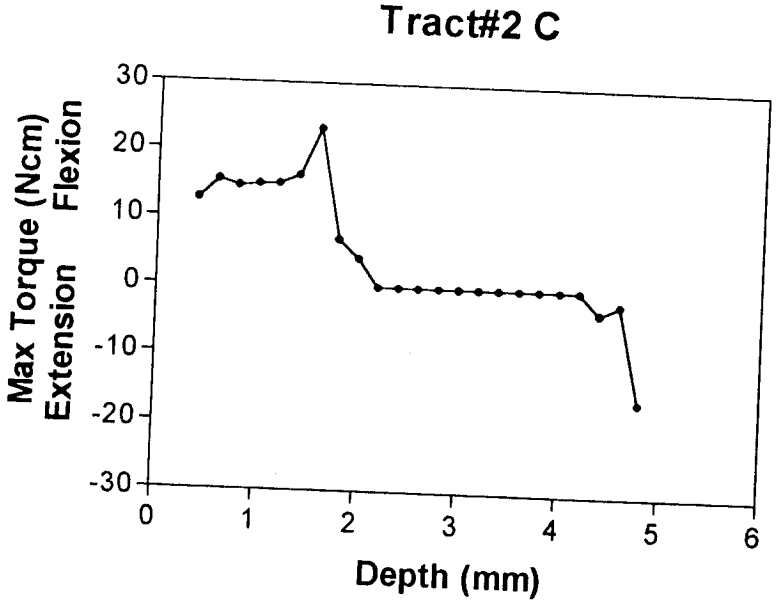

Tract#1 A
Max Torque (Ncm)
Extension Flexion
75
50
25
0
-25
-50
-75
0
1
2
3
4
5
Depth (mm)
Tract#1 B
Max Torque (Ncm)
Extension Flexion
50
25
0
-25
-50
0
1
2
3
4
5
Depth (mm)
Tract#1 C
Max Torque (Ncm)
Extension Flexion
50
25
0
-25
-50
0
1
2
3
4
5
Depth (mm)
Tract#1 D
Max Torque (Ncm)
Extension Flexion
50
25
0
-25
-50
0
1
2
3
4
5
Depth (mm)

Figure 1


Tract#2 A
Max Torque (Ncm)
Extension Flexion
100
75
50
25
0
-25
-50
-75
-100
0 1 2 3 4 5 6
Depth (mm)


Tract#2 B
Max Torque (Ncm)
Extension Flexion
180
120
60
0
-60
-120
-180
0 1 2 3 4 5 6
Depth (mm)


Tract#2 C
Max Torque (Ncm)
Extension Flexion
30
20
10
0
-10
-20
-30
0 1 2 3 4 5 6
Depth (mm)

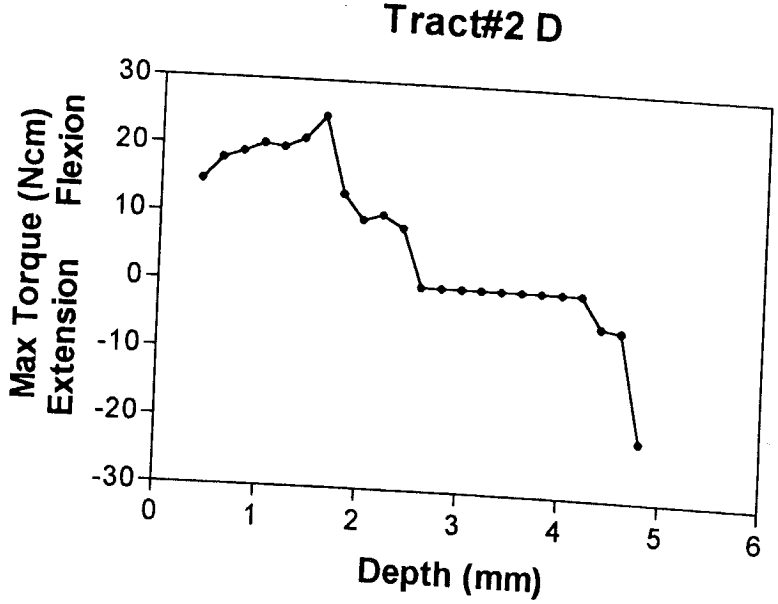
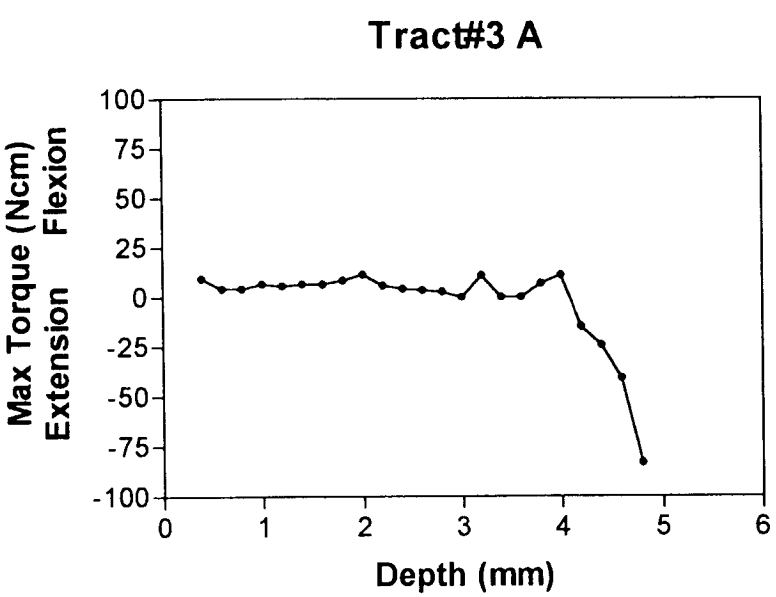
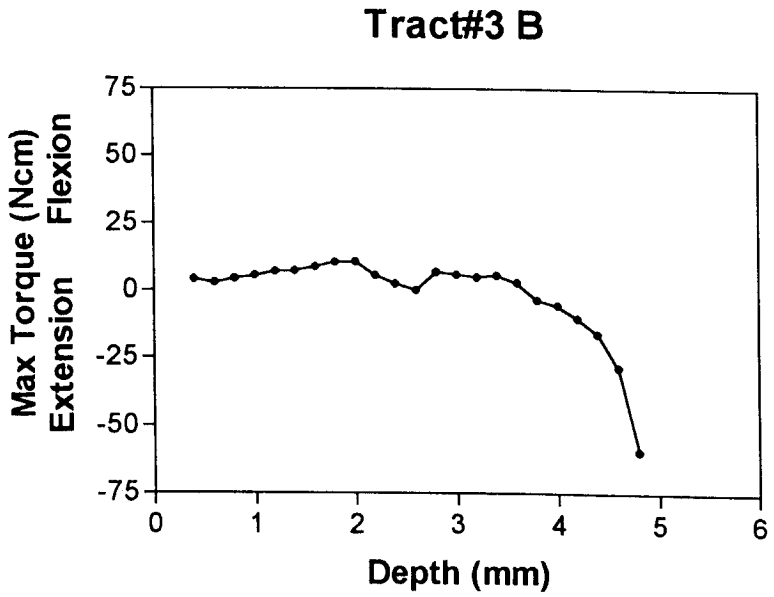
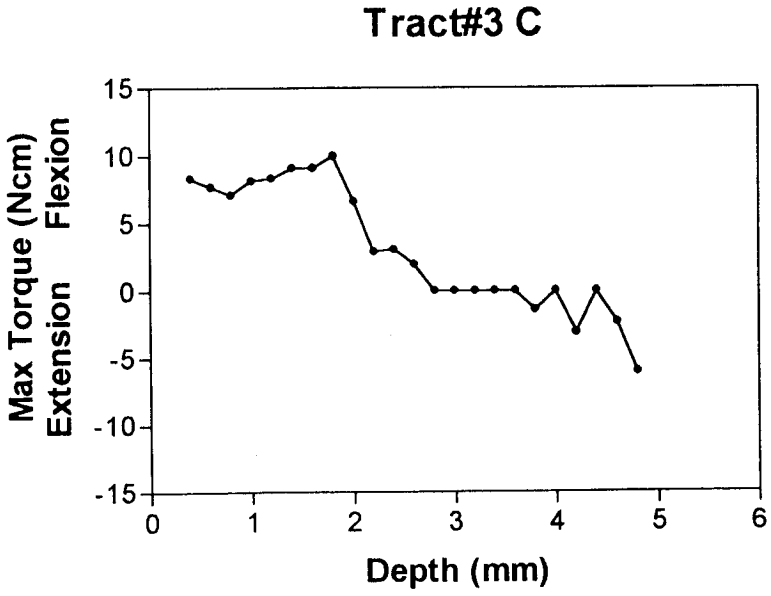

Tract#2 D
Max Torque (Ncm)
Extension Flexion
30
20
10
0
-10
-20
-30
0 1 2 3 4 5 6
Depth (mm)

Figure 2


Tract#3 A
Max Torque (Ncm)
Extension Flexion
100
75
50
25
0
-25
-50
-75
-100
0
1
2
3
4
5
6
Depth (mm)


Tract#3 B
Max Torque (Ncm)
Extension Flexion
75
50
25
0
-25
-50
-75
0
1
2
3
4
5
6
Depth (mm)


Tract#3 C
Max Torque (Ncm)
Extension Flexion
15
10
5
0
-5
-10
-15
0
1
2
3
4
5
6
Depth (mm)

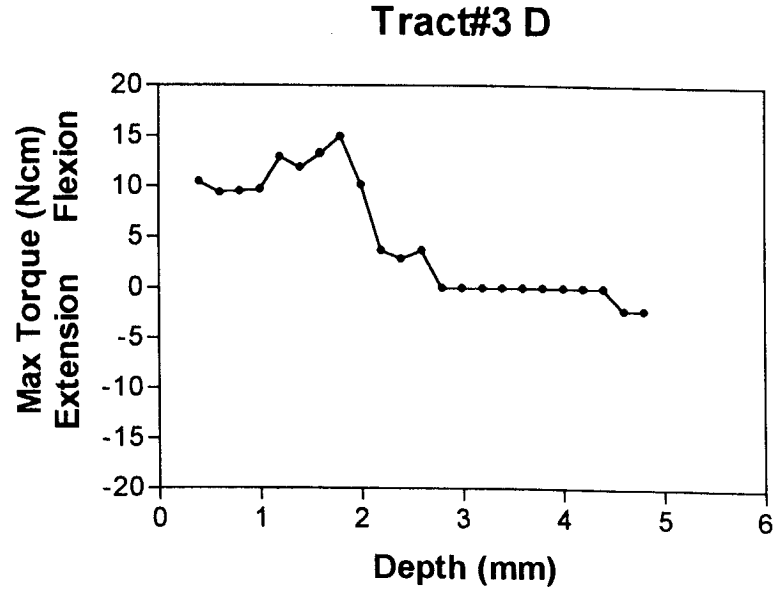

Tract#3 D
Max Torque (Ncm)
Extension Flexion
20
15
10
5
0
-5
-10
-15
-20
0
1
2
3
4
5
6
Depth (mm)

Figure 3

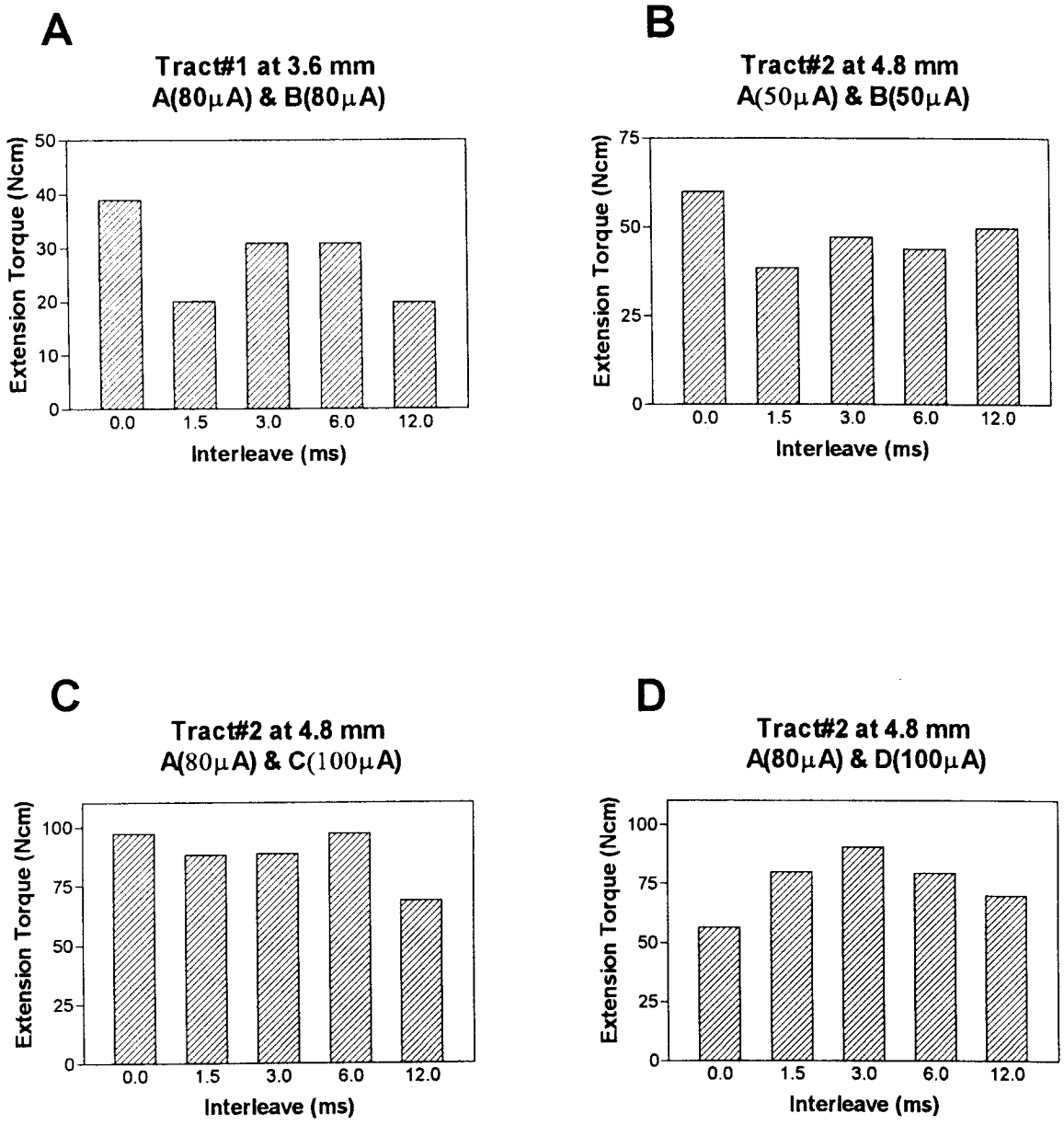

A
Tract#1 at 3.6 mm
A(80μA) & B(80μA)
Extension Torque (Ncm)
50
40
30
20
10
0
0.0
1.5
3.0
6.0
12.0
Interleave (ms)
B
Tract#2 at 4.8 mm
A(50μA) & B(50μA)
Extension Torque (Ncm)
75
50
25
0
0.0
1.5
3.0
6.0
12.0
Interleave (ms)
C
Tract#2 at 4.8 mm
A(80μA) & C(100μA)
Extension Torque (Ncm)
100
75
50
25
0
0.0
1.5
3.0
6.0
12.0
Interleave (ms)
D
Tract#2 at 4.8 mm
A(80μA) & D(100μA)
Extension Torque (Ncm)
100
75
50
25
0
0.0
1.5
3.0
6.0
12.0
Interleave (ms)

Figure 4

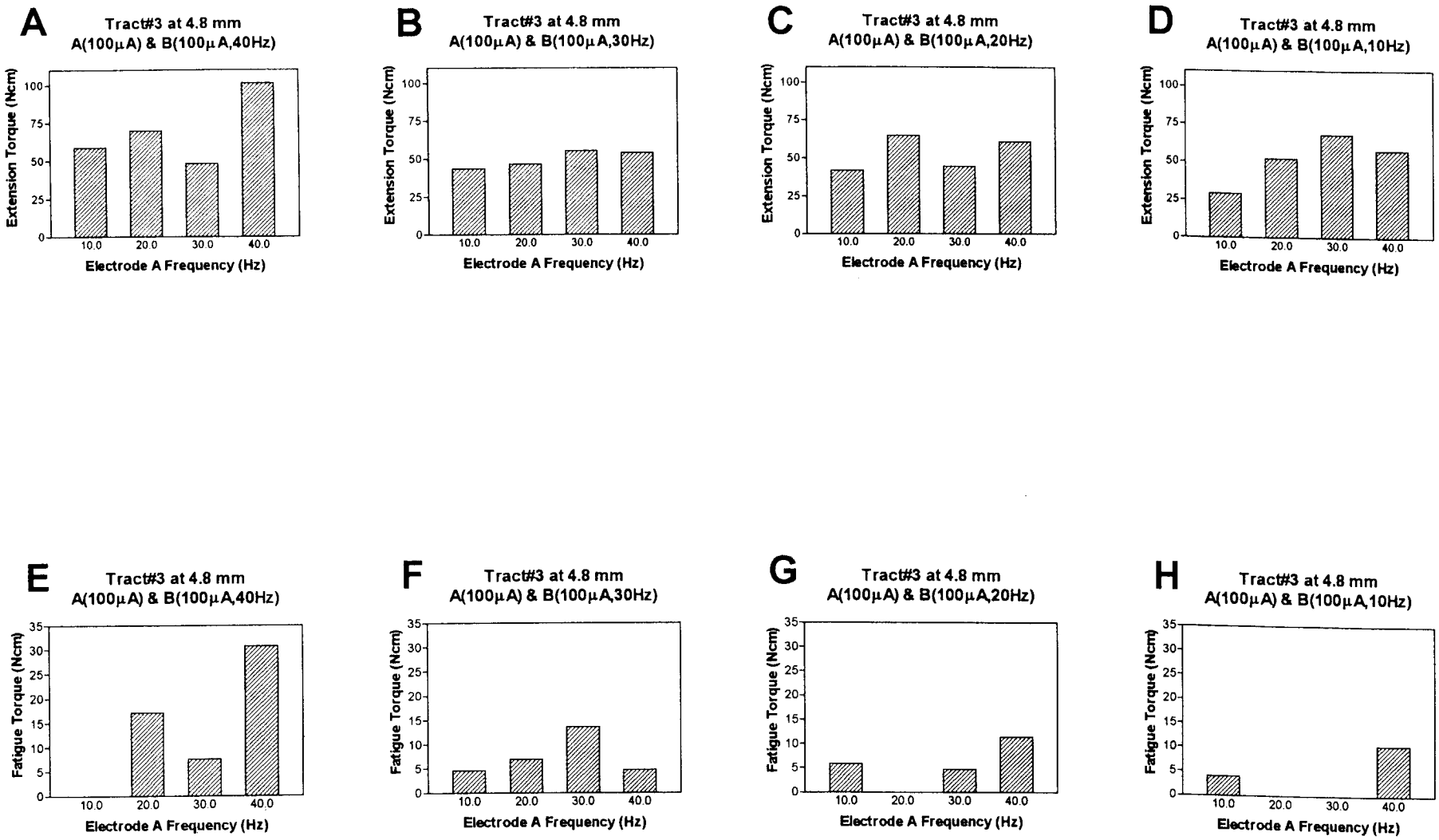
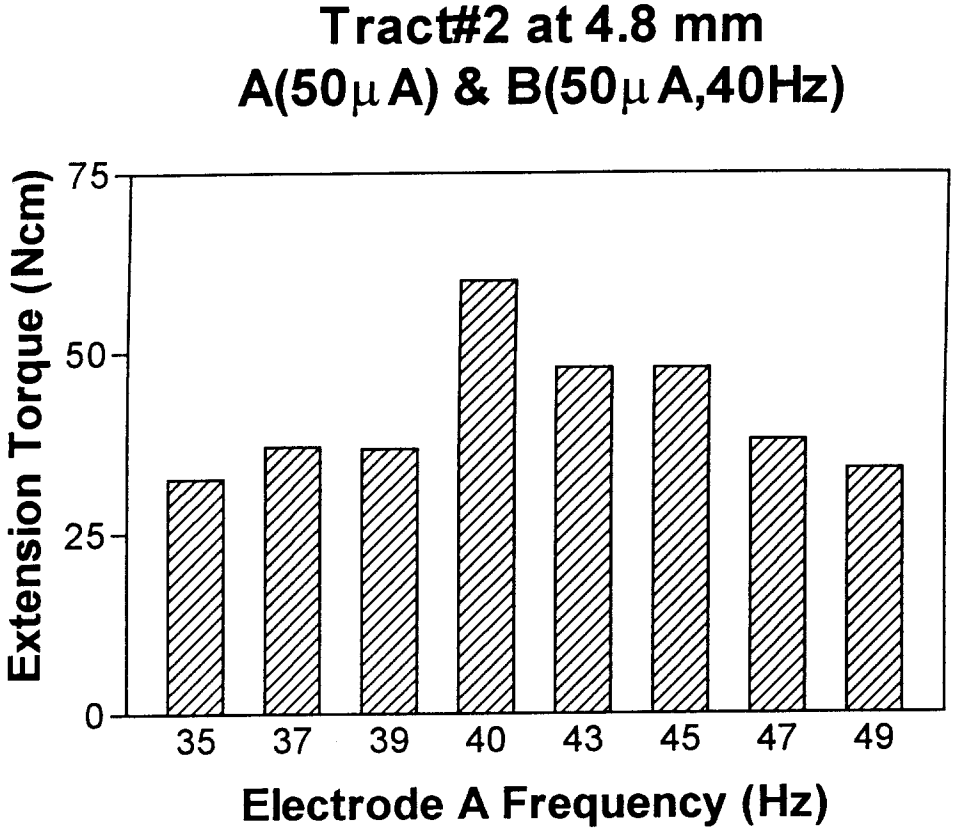

A
Tract#3 at 4.8 mm
A(100μA) & B(100μA,40Hz)
Extension Torque (Ncm)
100
75
50
25
0
10.0
20.0
30.0
40.0
Electrode A Frequency (Hz)
B
Tract#3 at 4.8 mm
A(100μA) & B(100μA,30Hz)
Extension Torque (Ncm)
Electrode A Frequency (Hz)
C
Tract#3 at 4.8 mm
A(100μA) & B(100μA,20Hz)
Extension Torque (Ncm)
Electrode A Frequency (Hz)
D
Tract#3 at 4.8 mm
A(100μA) & B(100μA,10Hz)
Extension Torque (Ncm)
Electrode A Frequency (Hz)
E
Tract#3 at 4.8 mm
A(100μA) & B(100μA,40Hz)
Fatigue Torque (Ncm)
35
30
25
20
15
10
5
0
10.0
20.0
30.0
40.0
Electrode A Frequency (Hz)
F
Tract#3 at 4.8 mm
A(100μA) & B(100μA,30Hz)
Fatigue Torque (Ncm)
Electrode A Frequency (Hz)
G
Tract#3 at 4.8 mm
A(100μA) & B(100μA,20Hz)
Fatigue Torque (Ncm)
Electrode A Frequency (Hz)
H
Tract#3 at 4.8 mm
A(100μA) & B(100μA,10Hz)
Fatigue Torque (Ncm)
Electrode A Frequency (Hz)

Figure 5


Tract#2 at 4.8 mm
A(50μA) & B(50μA,40Hz)
Extension Torque (Ncm)
75
50
25
0
35
37
39
40
43
45
47
49
Electrode A Frequency (Hz)

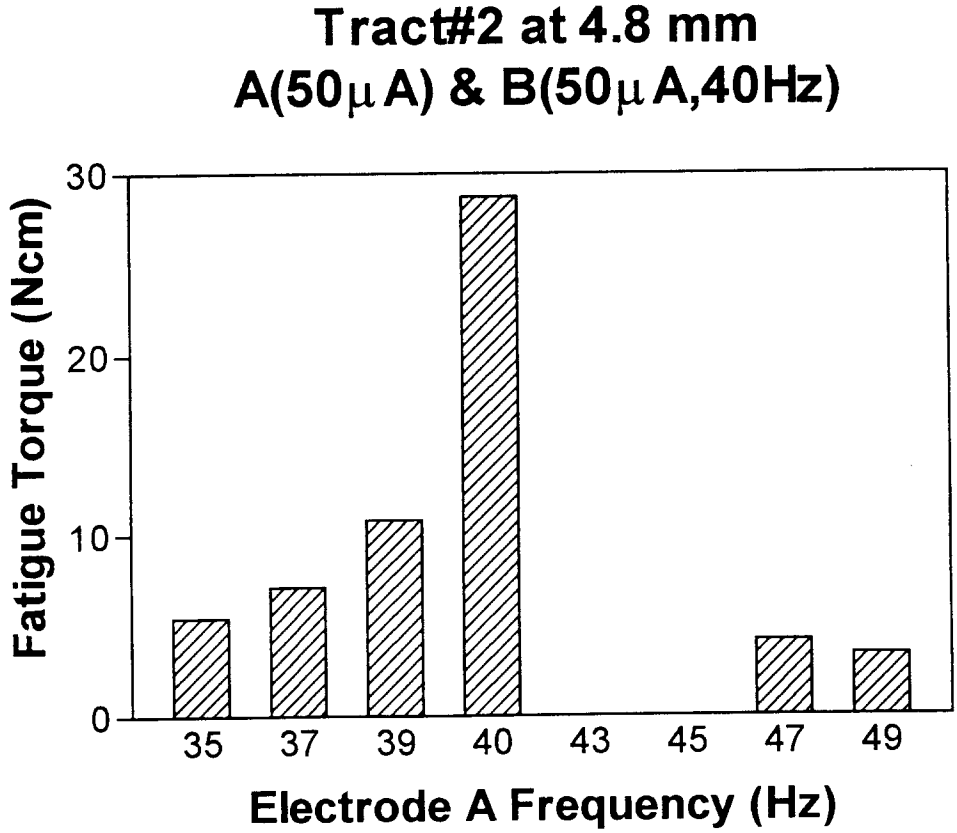

Tract#2 at 4.8 mm
A(50μA) & B(50μA,40Hz)
Fatigue Torque (Ncm)
30
20
10
0
35
37
39
40
43
45
47
49
Electrode A Frequency (Hz)

Figure 6

PRV into Colon

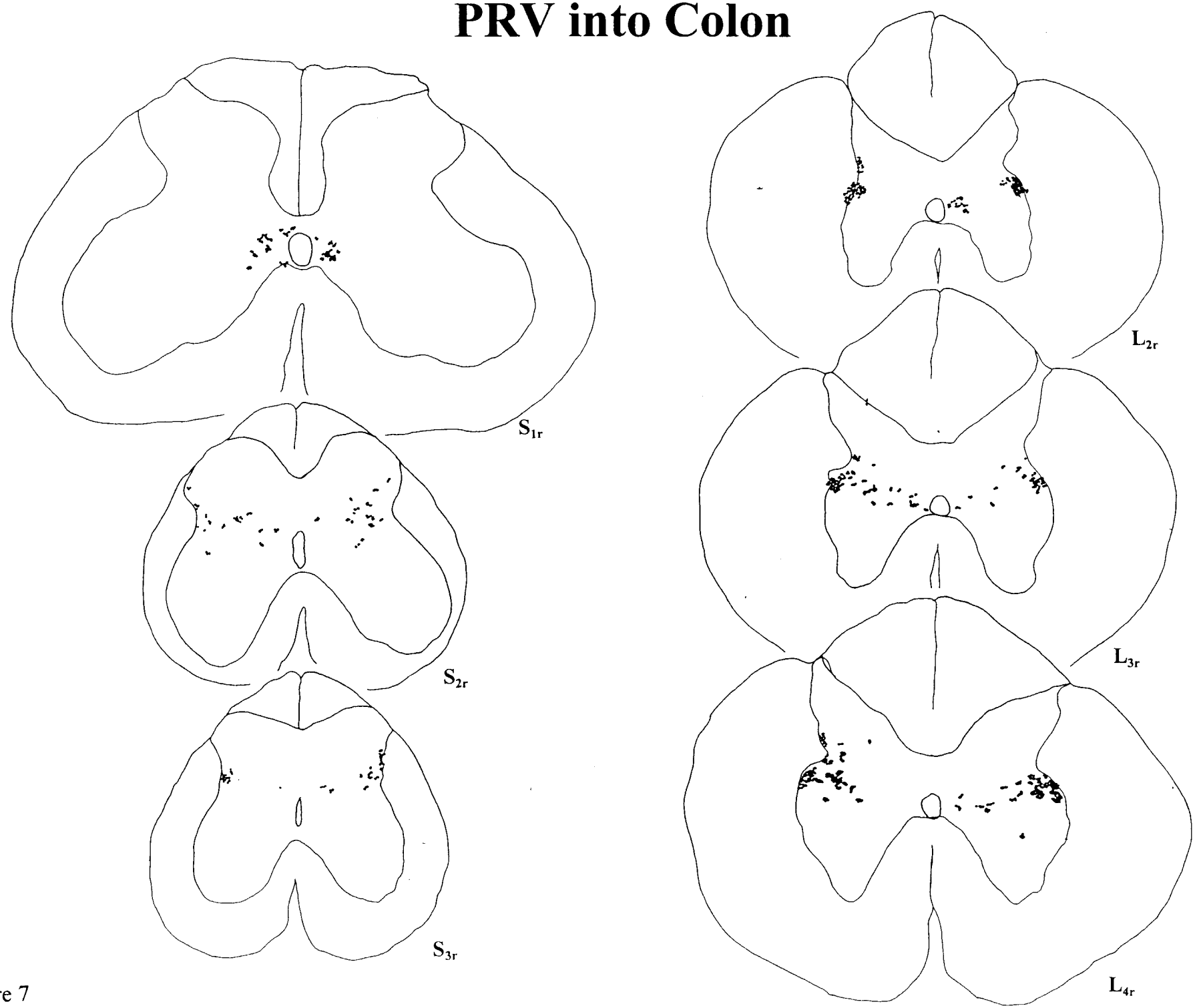


Figure 7